stabilizing their closed state. Furthermore, we have recently identified a series of residues in the C- and N-termini of Kir2.1 that are crucial for its sensitivity to cholesterol, suggesting a critical role for the cytosolic domain in cholesterol modulation of Kir channels.
Here we show that two cytosolic mutations, L222I and N251D, that are ~24Å apart from one another have similar effects on negating cholesterol and decreasing PI(4,5)P_2 sensitivity. Furthermore, both residues have similar effect on the open probability of the channel. These residues are allosterically coupled as the double mutant (L222I_N251D) reverts the effects of each single mutant. This result may be the major reason underlying the differences in cholesterol sensitivity and the strength of interaction with PI(4,5)P_2 of the four WT Kir2 channels, Kir2.1, Kir2.2, Kir2.3 and Kir2.4.
Moreover, our simulations suggest that the two residues are connected via two β strands through a critical salt bridge between K233 and D246. In agreement with our modeling results, the D246N mutation mimics each of the L222I and N251D mutations. This relationship demonstrates how the intricate arrangement of the cytsolic β sheets connects distant regions of the channel in a manner that enables control of channel gating and modulation.

129-Plat
Single Cell Dielectric Spectroscopy
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Pharmaceutical companies use a method known as patch clamping to quantify the potency of drugs by studying the effects of varied drug concentrations on membrane potentials. This particular method of patch clamping very accurately measures the effects and potency of pharmaceutical compounds as well as to study the range of side-effects caused by a drug. Patch clamping is a highly invasive and time consuming technique and has a high probability of destroying the cell. Dielectric spectroscopy is a non-invasive method that has acquired measurements comparable to those of patch clamping on a suspension of cells by placing them between a parallel plate capacitor and studying how the field changes with varying drug concentration. The field of microfluidics has brought about the possibility of scaling down this technique from a suspension of cells to single cells. A microfluidic flow chip was designed and fabricated to allow for cells in varied concentrations of drug solution to flow and pass through a parallel plate capacitor. Using numerical simulation, this capacitor was designed with guard electrodes to minimize fringing electric fields allowing for quick and accurate measurements comparable to patch clamp measurements.

130-Plat
Functional Assessment of Crystallization-Optimized G Protein-Coupled Receptors using Ion Channel-Coupled Receptors
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Ion Channel-Coupled Receptors (ICCRs) are artificial ligand-gated ion channels created by genetic fusion of G protein-coupled receptors (GPCRs) to a K+ inward rectifier channel (Kir6.2) such that the channel is a direct reporter of the receptor conformational changes. This concept has been validated with 4 prototypical GPCRs: the M2 muscarinic, the D2L dopaminergic, the β2 adrenergic and the opsin receptors (Moreau et al., Nature Nanotech 3:620 2008, Caro et al. PLoS ONE 6:e18226 2011, Caro et al. PLoS ONE 7:e43766 2012). Voltage-clamp recordings showed that ICCRs detect the agonist- and antagonist-bound states of the receptor via direct physical coupling. This GPCR-channel communication proceeds without any involvement of G proteins and the electrical signal amplitude is correlated with the ligand concentration.
The intrinsic instability of the GPCRs has proved a challenge to crystallographic studies. A successful approach, introduced in 2007 by Cherezov et al (Science. 318:1258) and subsequently applied to obtain 12 GPCR structures, consists in the insertion of the T4 phage lysozyme domain in the 3rd intracellular loop of the receptors. However, this modification abolishes G protein binding and prohibits related functional assays. Current characterization of crystallization-optimized GPCR(T4L) is performed by radiolabeled ligand assays or localized FRET techniques. Requiring no biochemical steps, ICCRs are an alternative tool to functionally characterize modified GPCRs that are unable to bind or activate G proteins and not amenable to most GPCR functional assays. We demonstrate here the validity of this tool with 3 different GPCRs (M2-muscarinic, β2-adrenergic and oxytocin receptors). The final application of this study would be the integration of this technology in the current crystallographic platforms dedicated to GPCR structure determination or for structure-function studies independent of G protein interaction.

131-Plat
High-Conductance K^+ Channels Show a Graded Sensitivity to Cell Volume Changes
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Slick (Slo2.1) and Slack (Slo2.2) Na^+/Cl^--activated high-conductance K^+ channels have been co-localized in CNS and are also probably present in the heart and epithelia. Furthermore, it has been found that these two channels can associate and form functional heteromers. A number of physiological processes, such as salt and water transport, central for neuronal activity, migration and apoptosis, involve changes in cell volume. We have previously shown that homomeric Slick channels are strongly regulated by changes in cell volume while the highly homologous channel Slack is totally insensitive. It is the aim of this work to evaluate if volume sensitive Slick subunits confer volume sensitivity to the otherwise insensitive Slack channels in tetrameric channels. For this purpose different configurations of Slick/Slack heteromeric channels were co-expressed with aquaporin1 in *Xenopus laevis* oocytes and cell volume changes of aprox. 5% were induced by exposure to hypotonic or hypertonic buffers. Whole-cell currents were measured by two electrode voltage clamp. Co-injections of Slick and Slack mRNA in different ratios (1:1-1:0.5-0.5:1) resulted in heteromeric channels sensitive to cell volume changes but to a smaller degree compared to homomeric Slick channels. Concatemeric Slick/Slack channels were also constructed and successfully expressed in oocytes. These chimeric channels showed, as co-injection experiments, higher whole cell currents than homomeric Slick or Slack channels and slower activation kinetics. Heteromeric channels resulting from the association Slick/Slack concatemers showed intermediate volume sensitivity between Slick and Slack channels, which seems to reflect the number of Slick subunits in the quatrameric channels. In conclusion, we have identified a heteromeric K^+ channel with graded sensitivity to small and fast changes in cell volume, a mechanism related with the number of volume sensitive subunits in the quatrameric channels.

132-Plat
Conformational Transition of KcsA Gating and the Mechanism of its pH-Dependence
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The bacterial K^+ channel KcsA has served as a prototypical system to study the architecture and the mechanism of gating of ion channels in response to proper stimuli. KcsA is activated in response to a drop in intracellular pH through the protonation of several His/Glu residues and the subsequent rearrangement of the helical bundle controlling the inner-gate opening. A constitutively open mutant channel (KcsA-OM) has been crystallized in several conformational states, each capturing a distinct degree of the inner-gate opening. However, due the heterogeneity of the structures and the use of antibodies, no firm conclusion could be made on the physiological relevance of the open conformation. In addition, due to the elimination of several charged residues, the mechanism of pH gating could not be fully determined by this particular mutant.
We have conducted both equilibrium and nonequilibrium driven MD simulations of the channel in membrane to probe the conformational variability of KcsA. When the pH-sensing residues are protonated in the simulations, the structure relaxes into an open conformation that resembles the crystal structure of KcsA-OM with second largest (23 Å) opening. The degree of opening captured in the simulations is consistent with that measured with EPR spectroscopy in the full-length KcsA, representing better the native state of the channel. Interestingly, the opening and closure of the cytoplasmic gate seem to be controlled by the competition between the protein-lipid interactions and several salt bridges between the channel's subunits. In particular, the neutralization of Glu118/Glu120 at low pH allows their entrance into the membrane, which permits the transmembrane helices surrounding the inner gate to tilt more and results in the opening of the channel.

133-Plat
Rate of Recovery from Slow Inactivation in K^+ Channels Controlled by Buried Water Molecules
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The transition of the selectivity filter of K+ channels between its two known important functional states, namely the conductive state and the slow

(c-type) inactivated state, is coupled to the opening and closing of the activation (intracellular) gate. Opening the activation gate by applying an external stimulus results in a transient current before the selectivity filter undergoes a spontaneous transition toward the non-conductive inactivated conformation that blocks the passage of ionic current through the channel. Only after closing the activation gate by removing the external stimulus will the selectivity filter return back to its original conductive conformation, resetting the filter so that it may once again pass current. While it is thought that recovery from the non-conductive inactivated state involves subtle conformational changes of the selectivity filter, the reason why this process can take up to several seconds, which is extremely slow on the molecular timescale, is not understood. Our results from a series of MD simulations reveal the selectivity filter is sterically locked in the inactive conformation for more than 15 microseconds by 12 buried water molecules, 3 for each subunit, that are strongly bound behind the filter. Even the presence of a few of these buried waters appears to lock the selectivity filter in the inactive conformation, blocking the filter from returning to a conductive conformation until the buried waters spontaneously vacate each subunit. Such an event would be rare, stretching the process of recovery to the timescale of seconds. To validate this mechanism, experiments were conducted where an osmotic stress was applied on the extracellular side of the channel to decrease the probability of waters occupying the cavities located behind the filter. As predicted, this accelerated the rate of recovery from slow inactivation.

Platform: Membrane Receptors & Signal Transduction I

134-Plat

Differences in the Allosteric Interaction between Agonists and GMP-PNP in Monomers and Oligomers of the M2 Muscarinic Cholinergic Receptor Fused to GαI1

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The nature of the signaling complex between G protein-coupled receptors (GPCRs) and G proteins remains unclear. GPCRs can exist as oligomers, yet monomers can activate G proteins when reconstituted in nanodiscs; the interaction with the G protein often is considered transient, yet some evidence suggests that the RG complex remains intact during signaling. Two classes of sites typically are observed in the binding of agonists to GPCRs in natural membranes, and guanylyl nucleotides such as GMP-PNP effect an apparent interconversion from higher (K_H) to lower affinity (K_L) without affecting affinity *per se*. We have examined three models of a stable RG complex in which the M_2 muscarinic receptor is fused to $G\alpha_{i1}$ via different linking sequences. Each fusion protein was expressed in *Sf*9 cells and characterized as an oligomer in digitonin-solubilized preparations, as a purified monomer in solution, and as an oligomer reconstituted in phospholipid vesicles. In the oligomeric state, the agonist oxotremorine-M recognized two classes of sites; GMP-PNP progressively increased the low-affinity fraction without affecting K_H or K_L. In the monomeric state, oxotremorine-M similarly recognized two classes of sites, most of which were of high affinity (F_H=71%); GMP-PNP progressively increased K_H, as expected for an allosteric interaction between two sites, without affecting K_L or F_H. The effect of GMP-PNP in monomers required the presence of DTT; the nucleotide-independent sites of low affinity may represent a subpopulation in which communication between the receptor- and α_{i1}-domains has been compromised. Only the oligomeric form of the fusion protein mimics the behavior of GPCRs in natural membranes, suggesting that signaling *in vivo* proceeds via a stable complex comprising multiple equivalents of receptor and G protein. (Supported by HSFO and CIHR)

135-Plat

Functional Comparison of Monomers and Tetramers of the M_2 Muscarinic Receptor

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M_2 muscarinic receptors were purified as monomers from *Sf*9 cells and reconstituted as monomers in nanodiscs and as tetramers in phospholipid vesicles. In the absence of G proteins, monomers in solution and in nanodiscs appeared homogeneous ($n_H \approx 1$), as revealed by the inhibitory effect of agonists and antagonists on binding of the antagonist *N*-[^{3}H]methylscopolamine. Reconstituted tetramers appeared homogenous to antagonists ($n_H > 0.80$) and heterogeneous to agonists ($n_H < 0.80$). The heterogeneity was modelled as two classes of sites ($\log\ldots IC_{50(High)}$, $\log\ldots IC_{50(Low)}$) and quantified as the product of the difference in affinity ($\Delta\log IC_{50}$) and the fraction of sites exhibiting higher affinity (F_H) (*i.e.*, $F_H\Delta\log\ldots IC_{50}$). The resulting values correlated with the corresponding values of $F_H\Delta\log\ldots IC_{50}$ and the intrinsic activities reported previously for binding and response, respectively, in natural membranes ($p < 0.0001$). In the presence of G proteins, reconstituted monomers and tetramers exhibited sensitivity to the guanyl nucleotide GMP-PNP. With tetramers, increasing concentrations of GMP-PNP caused an upward shift in the binding profile of the agonist oxotremorine-M, an effect that emerged from the model as an interconversion of labeled sites from high to low affinity with little or no change in affinity *per se*. With monomers, increasing concentrations of GMP-PNP caused a lateral rightward shift that emerged as an increase in the value of $\log\ldots IC_{50}$. The vertical shift displayed by tetramers mirrors the patterns observed previously in native membranes, while the lateral shift displayed by monomers seems to be artefactual. This observation, taken together with the correlation among agonists between efficacy and the heterogeneity of reconstituted tetramers devoid of G protein, suggests that oligomers—most likely tetramers—are the functional unit of the M_2 muscarinic receptor in nature. (Supported by HSFO and CIHR)

136-Plat

Enhanced Conformational Sampling of M_2 Muscarinic Acetylcholine Receptor for Designing Selective Allosteric Drugs

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Muscarinic acetylcholine receptors are members of the superfamily of G-protein coupled receptors (GPCRs) and play critical roles in both the central and parasympathetic nervous systems. They are important drug targets for the treatment of a spectrum of diseases including abnormal heart rate, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, Parkinson's disease and schizophrenia. Enhanced conformational sampling of M2 muscarinic receptor is achieved via accelerated molecular dynamics (aMD) simulations. The simulation output structures are clustered into representative conformers that are structurally distinct from each other. Potential allosteric binding sites are then identified by mapping the surface of the receptor conformers. This enables virtual screening of chemical compounds against the allosteric binding sites to discover receptor-selective drugs.

137-Plat

Novel Aspects of the Reversibility of the Antagonism at the Dopamine D2 Receptor by Antipsychotics

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All antipsychtics currently in clinical use are antagonists or weak partial agonists at the dopamine D2 receptor (D2R). Antipsychotic medication is associated with adverse effects such as extrapyramidal symptoms (EPS). The lower EPS liability of newer, so-called atypical antipsychotics, typified by clozapine, has been proposed to reflect their faster rates of dissociation from the D2R, as compared to older, typical antipsychotics such as haloperidol. This hypothesis has received increasing attention in recent years, and several pharmaceutical companies have endeavored to developed their own "fast off"-antipsychotics. However, previous studies have measured dissociation of radiolabeled antipsychotics or used modified G proteins to study receptor activation-induced calcium release, which confers certain limitations in terms of temporal resolution. We have examined antagonist dissociation in living cells, employing an assay based on the activation of G protein coupled potassium channels. This assay uses native G proteins and has higher temporal resolution than previous studies.

Our preliminary data suggest that there may be larger differences between different atypical antipsychotics than has previously been appreciated. Furthermore, the differences between atypical and typical drugs appear to relate mainly to the differential hydrophilicities of these drugs.

138-Plat

Supramolecular Architecture of Rhodopsin in Native Photoreceptors Revealed by Cryo-Electron Tomography

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Vision begins with the absorption of photons by rhodopsin, the visual pigment in photoreceptors. Rhodopsin belongs to the family of G protein-coupled receptors (GPCRs). Some family members form functional dimers or oligomers. Whether rhodopsin forms oligomers and whether these oligomers are functionally relevant is controversial [1-3]. We study rhodopsin organization in vitreous ultrathin cryo-sections of intact, dark-adapted photoreceptors by